DECLARATION I

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF:

JAMES FRANCIS ULRICH ET AL.

CASE NO.: BB-1057

SERIAL NO.: 08/196,622
FILED: FEBRUARY 15, 1994

GROUP ART UNIT: 1803
EXAMINER: E. VEITENHEIMER

FOR: CORN PLANTS AND PRODUCTS WITH IMPROVED OIL COMPOSITION

I hereby certify that this correspondenc being deposited with the United States Postal Se as first class mail in an envelope addressed to: C missioner of Patents and Trademarks, Washing D. C. 20231, on 5-12-95
Date of deposit
Angela D. Wronowic
Angela D. Wronowicz
Signature
May 12, 1995
Date of Signature

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

I, Kenneth J. Leto, declare as follows:

1. I am a citizen of the United States residing at 4712 Stonebridge Road, West Des Moines, IA 50265.

2. I am a graduate of the State University of New York at Albany, where I received a B.S. degree in 1973. I am also a graduate of the University of Missouri- Columbia, where I received a Ph.D. in Genetics and Plant Physiology in 1978. I was a postdoctoral fellow at the University of Illinois from 1978 to 1981. Since 1981 I have been employed by the E.I. du Pont de Nemours Company as, successively, a Member of the Research Staff in the Central Research and Development Department (1981-1986), and as a Section Research Biologist (1987 - 1990) and Senior Research Geneticist (1990 - 1993) and as a Senior Research Geneticist/Laboratory Manager in the Agricultural Products Department Biotechnology Research and Development Group.

3. Throughout my research career I have been engaged partly or wholly in the genetics, physiology and analysis of corn. During the past 6 years I have had technical responsibility for several corn nursery trait discovery programs, as well as direct responsibility for a seed trait analytical screen. Currently I have laboratory management responsibility for analysis of the composition of corn and soybean seeds and grain.

4. In the Office Action of 12/13/94 the Examiner asserts that "undue experimentation" would be needed to reproduce Applicant's invention for the production of high oil, high oleic grain by the method recited in Claim 1, and that the production of this grain be limited to exemplified materials. Applicants submit that the elevated grain oil contents recited in Claim 1 will be broadly obtainable when ASKC28 and derivatives are used to pollinate a large number of genetically distinct hybrid corn varieties. The data in Table 1 were derived by measuring the oil content by near infrared spectroscopy (BB1057 P. 20 L. 35 - P. 21 L. 13) of F1 kernels arising from pollinations between the high oil pollinator ASKC28 (Oil = 18.45% in Delaware) and a set of 51 low oil male sterile hybrid varieties. The inbred lines of corn which comprise these hybrids are all elite commercial lines, and many of the hybrids themselves are high yielding and are presently employed in commercial grain production. Where measured, the oil content of male fertile versions of these sterile hybrids did not exceed 5.07% oil in selfed grain, with the majority

falling below 4% in oil content. The pollinations were carried out by hand in corn nurseries, in three diverse locations: Franklin, Indiana, Williamsburg, Iowa, and Newark, Delaware. Data are presented below for the percent oil realized in F1 grain arising on the sterile hybrid varieties employed as female.

Table 1.

Average Per Cent Oil in F1 Kernels Arising by Pollination of Low Oil Hybrid Female Corn Plants with Two High Oil Pollinators in Three Locations, Summer 1992

Male Sterile Females	Hybrid Self Oil	---------ASKC28 as Pollinator--------- F1 Grain Oil		
		Indiana	Iowa	Delaware
LH119SDmswx LH82wx		11.79	9.47	10.2
LH202SDms X LH82	4.52	11.36	10.68	11.37
LH74wx X LH82wx		11.12	12.78	11.43
LHE136ELms X LH82	4.04	10.97	8.02	9
LHE136SDms X LH82	4.04	10.87	10.60	8.43
LH132ELms X LH85		10.65	9.59	10.25
LH74SDms x LH85	5.07	10.62	10.07	11.15
LH202SDms X LH181	3.89	10.15	9.64	7.89
LH74SDms X LH82	4.06	10.07	10.33	10.5
LHI99 X LH60		9.95	8.15	8.29
LH202SDms X LH163	4.19	9.95	9.25	9.29
LH132SDms X LH128		9.93	7.71	8.32
LH74SDms X LH172	4.14	9.93	9.43	10.29
LH202SDms X LH127	4.28	9.84	9.84	9.35
LH206SDms X LH82	4.10	9.80	9.80	10.08
LH74SDms X LH213		9 73	9.39	10.14
LH19SSDms X LH213	3.82	9.72	8.16	9.07
LH132SDms X LH172	3.85	9.61	9.07	10.1
LH132SDms X LH213	3.86	9.59	8.92	8.27
LH119wx X LH59wx		9.49	9.71	7.94
LH132SDms X LH181	3.53	9.45	7.73	7.48
LH192SDms X LH82	4.81	9.45	9.63	9.05
LH132ELms X LH123		9.32	8.36	6.4
LH74SDms X LH163	3.95	9.25	6.85	10.5
LH195VGms X LH213	3.82	9.10	7.40	7.93
LH132SDms X LH157		8.97	8.59	7.8
LH119wx X LH51wx		8.95	8.43	6.43
LH132SDms X LH127	3.9	8.95	8.45	7.33
LH192SDms X LH127		8.87	9.37	8.02
LH74SDms X LH62		8.83	8.41	9.09
LH195SDms X LH212	3.12	8.75	8.23	7.54
LH132SDms X LH123		8.74	8.04	6.54
LH119SDms X LH163		8.72	8.72	8.05
LH192SDms X LH123		8.68	7.94	7.47
LH195VGms X LH212	3.12	8.68	7.18	8.01
LH74SDms X LH181		8.64	8.84	7.7
LH74SDms X LH123		8.61	8.59	9.05
LH132SDms X LH211	3.77	8.60	7.54	7
LH206SDms X LH213	3.58	8.59	7.77	8.22
LH199 X LH212	3.47	8.55	8.35	7.27

Table 1 (continued)

Average Per Cent Oil in F1 Kernels Arising by Pollination of Low Oil Hybrid Female Corn Plants with Two High Oil Pollinators in Three Locations, Summer 1992

Male Sterile Females	Hybrid Self Oil	---------ASKC28 as Pollinator--------- F1 Grain Oil		
LH209SDms X LH61		8.27	7.15	7.09
LH206SDms X LH61	3.32	8.21	6.81	7.77
LH74SDms X LH216	3.66	8.21	7.77	9.07
LH74SDms X LH61	3.70	8.14	7.54	8.57
LH195SDms X LH210	3.79	8.08	7.60	7.03
LH206SDms X LH181	8.07	8.21	8.38	5.16
LH192SDms X LH211	3.33	8.01	8.09	7.26
LH195VGms X LH210	3.79	7.95	7.05	7.01
LH205SDms X LH62		7.63	7.01	8.23
LH206SDms X LH62	3.26	7.19	7.01	8.25
LH205SDms X LH61		7.12	8.10	7.59
Range in Oil (%)	5.07-3.12	11.8-7.1	12.8-6.8	11.4-6.4
Average Oil (%)	3.86	9.21	8.58	8.52
Standard Deviation	0.45	1.04	1.18	1.30

The data clearly establish that ASKC28 pollen confers high oil to grain across a large set of hybrid corn plants used as female, and that ASKC28 pollen exhibits a xenia-like effect for oil quantity. It is therefore expected that high oil derivatives of ASKC28, such as ASKC28OL exemplified in the specification (Example 1, Table 1), will broadly confer high oil to grain produced on a wide variety of grain parents as well.

5) To further establish that the instant invention is not limited to the direct use of exemplified materials only, data are included below to illustrate that B73OL and AEC27-2OL can be used effectively in a breeding program to increase the oleic acid content of many different corn inbreds. It is reasonable to expect that these inbreds, when finished, will combine to produce high yielding corn hybrids with a high oleic acid content. Male sterile versions of these inbreds can then be pollinated by ASKC28OL or similar high oil derivatives to produce the grain described in the instant Invention.

The data in Table 2 are taken from a partially completed backcross breeding program. Backcrossing is a conservative breeding method which is most often used to introduce simply inherited, highly heritable traits into existing agronomically elite inbred lines. In a typical backcrossing program involving a quality grain trait (i.e. a trait which influences the composition of a corn kernel, such as wx), one or a series of varieties containing a quality grain trait are crossed to a series of elite inbred lines, which are termed "recurrent parents". The progeny of these crosses are again crossed back to their respective recurrent parents, and this cycle is repeated typically 5 to 8 times. During this process the quality grain trait is maintained in each backcrossing project by visual or other selection, and the average nuclear genetic composition of each emerging inbred becomes closely similar to that of the elite recurrent parent. The result is the production of a series of elite inbred lines which express the newly introduced grain quality trait and in all other respects very closely resemble the plant type and combining characteristics of their respective recurrent parents. Finally, these finished inbreds are selfed and homozygous individuals selected so the quality grain trait is uniformly expressed in subsequent seed increases.

B73 as a line has given rise to a large number of elite female corn inbreds widely employed in commercial production today. Further, B73 is closely related to several of the inbreds employed as recurrent parents in Table 1. This further increases the probability that elite female, and possibly male, inbreds will be recovered from backcrossing projects employing B73OL as a source of the high oleic trait. While not as elite as B73, AEC272 is a well adapted experimental high oil male inbred and performs reasonably well in "prototype" high oil hybrids, suggesting that male, and perhaps female, inbreds derived from AEC272OL after backcrossing should again yield agronomically elite inbreds.

The data in Table 2 give the range of oleic values observed in the selfed progeny of a number of backcross conversion projects in which AEC272OL or B73OL were used as donors of the high oleic oil trait. The level of oleic acid in these projects was determined by selfing partially backcrossed lines during each backcrossing cycle, bulking the resultant kernels (termed "S1") individually by ear, and measuring the fatty acid composition of a representative sample of kernels by gas chromotography using a slight modification of the extraction and analytical methods described in Example 1 of BB1057. We expect the genes causing high oleic acid content to segregate in these populations and thus expect a broad range of oleic acid content across S1 ears in projects that continued to carry the high oleic traits. In contrast, if the high oleic acid trait was either not expressed in a particular genetic background or was lost by faulty selection during the backcrossing process, we would expect a typically narrow range of oleic content in the kernels from selfed ears, as is typical of inbred lines of corn.

The range of oleic content of each of the recurrent parent inbreds was typically about 5%.

Table 2.

Per Cent Oleic Acid in Oil Extracted from Lines undergoing Backcross Conversion Grown in 1994 in Molokai, Hawaii

Recurrent Parent	Oleic Source	Backcross Generation (n)	--% Oleic in BC(n) S1--- Range	Average	Observations
LH59	AEC272OL	4	44-20	32.4	20
LH60	AEC272OL	4	53-31	41.9	8
LH61	LH105 X B73OL	4	33-27	30.4	4
LH74	LH105 X B73OL	4	44-25	32.0	11
LH82	AEC272OL	4	42-21	26.8	14
LH85	AEC272OL	3	45-25	33.0	19
LH132	B73OL	3	57-23	37.7	12
LH132	LH105 X B73OL	4	40-23	28.3	18
LH145	LH105 X B73OL	4	54-22	31.8	16
LH146	B73OL	4	33-22	26.5	11
LH150	LH105 X B73OL	3	------	26.4	1
LH163	AEC272OL	4	43-29	34.0	12
LH168	LH82(4) X AEC272OL	0	57-24	42.4	11
LH169	LH82(4) X AEC272OL	0	53-24	35.9	13
LH172	LH82(5) X AEC272OL	0	48-20	31.5	15
LH172	LH105 X B73OL	3	39-23	33.1	6
LH185	LH59(5) X AEC272OL	0	43-22	31.1	8
LH186	LH59(5) X AEC272OL	0	33-20	25.7	10

Table 2 (continued)

Per Cent Oleic Acid in Oil Extracted from Lines undergoing Backcross Conversion Grown in 1994 in Molokai, Hawaii

Recurrent Parent	Oleic Source	Backcross Generation (n)	--% Oleic in BC(n) S1--- Range	Average	Observations
LH192	LH105 X B73OL	4	58-37	43.8	16
LH192	AEC272	4	61-37	46.2	14
LH195	LH105 X B73OL	3	55-27	32.3	18
LH197	LH105 X B73OL	4	62-27	39.0	19
LH198	LH105 X B73OL	3	50-37	44.0	4
LH199	132(5) X B73OL	0	40-29	34.4	2
LH200	LH105 X B73OL	4	43-26	34.6	5
LH206	LH150(5) X LH150 X B73OL	0	43-24	31.4	10
LH206	LH105 X B73OL	4	47-30	38.1	9
LH211	LH105 X B73OL	4	35-21	26.1	8
LH212	LH216(5) X AEC272OL	0	31-20	25.1	10
LH213	LH216(5) X AEC272	0	45-25	34.4	13
LH213	LH18 X B73OL	3	58-33	46.1	8
LH216	AEC272OL	4	36-24	30.5	6
LH218	LH216(5) X AEC272OL	0	31-18	24.3	11
LH219	LH216(5) X AEC272OL	0	34-25	28.8	9
LH223	B73OL	4	45-28	34.2	10
LH225	LH18 X B73OL	4	51-26	34.3	15

Out of the 36 backcross projects presented in Table 2, 17 exhibited a range of oleic acid contents of 20 percentage points or greater, while 16 exhibited a range of oleic acid content of 11 percentage points or greater. Of the remaining projects LH150 returned only one ear during this cycle and hence failed to show a range of segregation, and the oleic trait may have been lost during the course of the LH61 and LH219 backcross projects. Overall, these results indicate that B73OL and AEC272OL can be used as effective donors of the high oleic acid trait, and that the high oleic trait is expressed at sufficiently high levels in a number of genetic backgrounds to allow simple, effective selection during backcross breeding.

6) Oil extracted from B73OL and AEC272OL kernels typically exhibit oleic acid levels of 60%. These levels of oleic acid are generally not seen in Table 2 because none of the S1 ears examined are expected to be homozygous for the oleic genes present in either AEC272OL or B73OL. To gain an estimate of the final oleic acid level which may be achieved in finished lines after backcross conversion, plants from the second backcross generation (BC2) from several backcross projects were self pollinated to yield BC2S1 kernels. Ears bearing BC2S1 kernels exhibiting elevated oleic contents when bulked were replanted in a field in Newark, Delaware in the summer of 1994 and resultant plants were self pollinated to yield BC2S2 kernels. The oleic values determined as described in Table 1 from bulks of kernels taken from single ears bearing BC2S2 kernels are presented in Table 3. It was expected that a proportion of these BC1S2 plants would be homozygous for the high oleic gene present in B73OL and AEC272OL and that kernels obtained from BC2S2 ears from these homozygous plants would be uniformly high in oleic acid content. Kernels produced on the remainder of the BC2S2 ears would either exhibit oleic acid levels typical of corn inbreds or would contain a mixture of grain types. The upper range of oleic acid content seen in BC2S2 ears should thus be indicative of

the expression of kernels uniformly expressing the high oleic trait. Since approximately 87% of the nuclear genome of BC2S2 kernels should be derived from the recurrent parent, these oleic levels should be generally representative of the oleic acid content of oil extracted from seeds of the finished inbreds when these backcrossing projects are completed.

Table 3
Per Cent Oleic Acid in Oil Extracted from BC2S2 Kernels Bulked By Ear Produced in Newark, Delaware During Summer 1994

Recurrent Parent	Oleic Source	---Per Cent Oleic, BC2S2 --- Range	Average	Observations
LH59	AEC272OL	64-24	40.4	49
LH60	AEC272OL	59-43	43.6	39
LH61	LH105/B73OL	62-24	39.6	44
LH74	LH105/B73OL	62-26	40	6
LH82	AEC272OL	58-23	38.1	55
LH85	AEC272OL	60.5-23.4	36.7	51
LH132	LH105/B73OL	62.5-22.6	40.22	51
LH132	B73OL	58.4-21.6	39	50
LH145	LH105/B73OL	66.5-21.5	37.3	43
LH146	B73OL	63.7-22.3	40.8	48
LH150	LH105/B73OL	53-27	39.1	15
LH163	AEC272OL	57.6-25.5	39.5	45
LH172	LH105/B73OL	59.7-23.9	36.4	34
LH192	LH105/B73OL	70-32	52	46
LH192	AEC272OL	70.4-24.9	53.2	31
LH195	LH105/B73OL	60.5-22.3	35.6	55
LH197	LH105/B73OL	59.9-24	40.7	56
LH198	LH105/B73OL	67.5-2.6	42.6	53
LH200	LH105/B73OL	62-25.3	39.9	56
LH206	LH105/B73OL	50.3-23.6	35.6	44
LH211	LH105/B73OL	55.2 - 23.8	33.8	31
LH212	LH105/B73OL	62.1-22.1	41.8	34
LH213 (?)	LH18 X B73OL	55.8-28.4	39.6	25
LH216	AEC272OL	46.6-24.7	32.2	33
LH223	B73OL	48.3-26.4	36.2	28
LH225	LH18 X B73OL	48.3-29.7	41.3	20

Of the 26 backcross projects examined in Table 3, 19 returned ears with kernels containing oil with an oleic acid content of 57% or greater. Four of these projects contained oil with an oleic acid content of 65% or greater, and the two backcross projects involving LH192 as recurrent parent returned ears whose kernel oil contained 70% oleic acid. Table 3 further illustrates that it is possible to recover high oleic segregants in the LH61 project and hence the failure to recover high oleic LH61 segregants in Table 2 is more likely due to missed selection during the latter stages of the backcrossing program rather than by any "suppressive" effect of the LH61 background.

7) It is reasonable to expect that both AEC272OL and B73OL will be effective in producing numerous inbred lines of corn by the backcross method of breeding In most cases

these inbreds will produce kernels whose oil will contain approximately 60% oleic acid. In some genetic backgrounds the final percentage of oleic acid is likely to approach 70%.

It is also likely that breeding methods other than those employed during backcross conversion, such as exemplified in WO 92/01367 will also be effective in producing new inbreds containing oil which in many cases contains approximately 60% oleic acid, because the inheritance of the oleic trait from these sources is simple, highly selectable, and does not exhibit a high degree of either genotypic or environmental sensitivity. B73 has favorable agronomics and has been used to derive a number of currently high performing female inbreds by methods other than backcross conversion. AEC272 is currently being explored for it's potential to produce high performing male inbreds in non backcrossed breeding projects.

Since the oil of selfed kernels from the hybrid B73OL X AEC272OL is itself 60% oleic acid, it is very likely that inbreds derived from either B73OL or AEC272OL can by combined in hybrid combination to produce hybrid corn grain containing oil with approximately 60% oleic acid content.

8) Example two in the Specification teaches the production of grain containing oil with an oleic acid content of approximately 60% when utilizing various combinations of AEC272OL, B73OL, ASKC28OL, and standard corn inbreds. The data presented in this example suggest that similar combinations made between suitably selected inbreds derived from either AEC272OL or B73OL and the high oil, high oleic pollinator ASKC28OL or derivatives will similarly produce grain containing approximately 6.5 to 10% oil, which oil has a content of approximately 60% oleic acid. Thus the Invention is not limited to the specified materials per se, but can reasonably be expected to be practiced using a wide range of corn inbreds derived in part from B73OL and AEC272OL following selection of oleic content and agronomic performance.

9) Applicants also show that unrelated high oleic corn varieties can combine with AEC272OL and B73ol to produce high oleic corn kernels and grain. In Table 3 of this Declaration AEC272OL (60% oleic) backcrossed to the unrelated inbred LH192 (approx. 40% oleic)
is shown to give transgressive segregants as high as 70.4% oleic in bulked BC2S2 kernels. Applicants exemplify that the hybrid between B73ol (60% oleic) and the unrelated inbred LH60 (35-40% oleic) yields grain upon sib pollination with an oleic content of 45-50%. This hybrid when employed as female and directionally pollinated by ASKC28OL yields grain with an oleic content of 56.8% (Specification, P. 30 L. 7-21. Thus, unrelated oleic sources can combine to produce high oleic kernels in backcross breeding projects, in grain produced on hybrid corn plants by conventional methods of sib pollination, and also by directional pollination of female corn plants by male corn plants by the methods recited in Claim 1 of BB1057.

10) I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with that knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Kenneth J. Leto

Kenneth J. Leto

5/10/95

Date